

REMARKS

STATUS OF THE CLAIMS:

Claims 1-22 are pending. Claims 1 and 10-22 are rejected under §112, second paragraph. Claims 1-22 are further rejected as obvious.

The drawings are objected to as informal. Applicants will supply formal drawings after the Examiner has determined that the application has claims that are allowable.

Claim 1 has been amended to recite amplification systems which produce DNA. Support for this amendment is found at pages 11-12 where different amplification systems are described with produce both DNA and RNA.

THE INVENTION:


This invention provides for a means of simultaneously amplifying and detecting amplified PCR target nucleic acid. Simultaneous amplification and detection is made possible through the use of intercalation agents which give a photodetectable signal when bound to duplex nucleic acid. Through the use photodetectors it is possible to measure the amplification of nucleic acid while the PCR reaction is proceeding.

SECTION 112:

The original claims 1, 10, 11 and 17 were rejected as indefinite under §112. Claim 1 was rejected for use of the phrase "said method comprising". Claim 1 has been amended according to the Examiner's suggestion.

Claim 10 was amended to correct the grammatical concerns of the Examiner. Specifically, the reference to "before and after PCR" has been deleted and reference to "during amplification" has been inserted.

Original claim 11 was rejected as indefinite for recitation of "a mixture that comprises a PCR." Claim 11 was amended as suggested by the Examiner.



Applicants believe that they have fully addressed the §112 concerns of the Examiner. Reconsideration of the claims in view of the amendments is requested.

SECTION 103:

The Examiner has rejected claims 1-22, under 35 USC §103, as being unpatentable over Sutherland et al. in view of Mullis et al. Sutherland is cited as disclosing "methods for the use of fluorescent dyes including in particular ethidium bromide for measurement of polymerization of nucleic acids during PCR amplification...that these dyes can be provided directly in the PCR reaction and that the dyes have a greater fluorescence when bound to double stranded DNA than when either bound by single stranded DNA or unbound." Mullis cited as disclosing PCR.

Applicant will overcome the *prima facie* case of obviousness by argument and by directing the Examiner's attention to surprising results. There are four bases for rebutting the Examiner's conclusion regarding the obviousness of the claims. In brief, the Examiner has misinterpreted the Sutherland reference, there is no motivation to combine intercalating agents into PCR mixtures, there was literature teaching away from the use of fluorescent intercalating agents in PCR, and finally, applicants surprisingly demonstrate with ethidium bromide [EtBr] that there is no significant inhibition of PCR by intercalating agents at the recited concentrations.

(1) The Examiner has misread Sutherland et. al.

The Examiner cites Sutherland as teaching the use of EtBr during PCR. Applicants have reviewed Sutherland and cannot find any example or teaching that suggests the use of EtBr during PCR. The Sutherland disclosure measures polymerase activity without the repeated thermal cycling that defines the exponential amplification of PCR. Applicants have noted that a PCR mixture was used in Sutherland's example 6; but, no PCR was conducted. If the applicants' have misread Sutherland, the Examiner is asked to direct applicants' attention to a specific teaching of adding

EtBr during PCR. If the applicants' have correctly interpreted Sutherland, the Examiner is asked to reconsider her rejection and either withdraw the rejection or clearly set forth her basis for maintaining the rejection.

(2) The combination of references fails to suggest the introduction of intercalating agents into a PCR reaction mixture.

The consistent criterion for determination of obviousness is whether the prior art would have suggested to one of ordinary skill in the art that a claimed invention should be carried out. More specifically, the Federal Circuit's standard for combining references to support an obviousness rejection requires a clear suggestion in the art to combine.

The Patent Board of Appeals recently stated that examiners must set forth their reason for combining art in a clear and convincing manner. See *Ex parte Clapp*, 227 USPQ 972 at 973 where the Board states:

Presuming arguendo that the references show the elements or concepts urged by the examiner, the examiner has presented no line of reasoning...as to why the artisan viewing only the collective teachings would have found it obvious to selectively pick and choose the elements and/or concepts from the several references relied on to arrive at the claimed invention....To support the conclusion that the claimed combination is directed to obvious subject matter, either the references must expressly or impliedly suggest the claimed combination or the examiner must present a convincing line of reasoning as to why the artisan would have found the claimed invention to have been obvious in light of the teachings of the references.

The Sutherland and Mullis references are without an express suggestion to combine PCR with intercalating agents and the applicants find the Examiner's rejection devoid the *Clapp* reasoning. Her sole reasoning for combining Sutherland with Mullis is at page 4 of the Office Action where she states:

...it would have been obvious to one of ordinary skill in the art to use the method of detecting polymerization by use of fluorescent dyes during a PCR amplification to detect the target nucleic acid of the amplification reaction...

This is a subjective conclusion. There is no *Clapp*-type reasoning articulated by the Examiner. Applicants respectfully ask, what is the motivation to introduce fluorescent intercalating agents into the Mullis PCR reaction mixture before amplification? Sutherland discloses use of such agents in a single step primer extension conducted without repeated thermal cycling. Mullis teaches the detection of PCR product after amplification is complete. There is simply no suggestion to use the Sutherland reagents for measuring amplification during PCR as taught by Mullis.

The Examiner's conclusion that the invention would have been obvious to one of skill is apparently based on hindsight. The Examiner appears to have focused on her belief that one of skill, with knowledge of the cited art, would have considered the introduction of fluorescent intercalating agents during PCR to be operable. But this is not the test for *prima facie* obviousness. The true test is whether the references motivate the combination - not whether one of skill would have thought it operable, once described by a third party. Furthermore, the Examiner cannot rely upon the applicants' teaching for such motivation.

Having explained that the combination of Sutherland and Mullis fail to motivate the claimed combination and that the Examiner's rationale for combining the references fails the Patent Board of Appeals requirement for a "convincing line of reasoning", applicants submit that the rejection is improperly based and should be withdrawn.

(3) When all the references are viewed together, there is a suggestion teaching away from the invention.

As the Examiner is aware, a *prima facie* case of obviousness can be overcome by presenting evidence in the art that teaches away from the claimed invention. To determine whether an invention is obvious, patent examiners must look at the references as a whole and not ignore references or statements which teach away from our conclusions. *Akzo N.V. v USITC*, 1 USPQ

2d 1240 at 1246 (CAFC 1986). And where such evidence is presented by an applicant, the examiner must revisit his or her rejection anew and not judge whether the additional evidence merely rebuts his or her original conclusion. For as the Patent Board of Appeals stated in *Ex parte Ohsaka*:

...when prima facie obviousness has been established and evidence is submitted in rebuttal, the decision-maker must start over...An earlier decision should not, as it was here, be considered as set in concrete...[T]he examiner must consider all the evidence anew. 2 USPQ 2d 1461, 1462 (PTOBPA&I 1987).

Applicants direct the Examiner to two references, AA and AB included with the 1449 form supplied with this Amendment. Both references document that those of skill understood that intercalating agents would inhibit DNA polymerase activity. Reference AA is Kirk-Othmer Encyclopedia of Chemical Technology 3rd Ed. and states at 3:907:

Studies of the interaction of model compounds such as dyes and also drugs, such as actinomycin and ethidium bromide, have shown that these molecules are complexed or intercalated within the helix of the polynucleotide strands which in the case of the nucleic acid, effectively inhibit replication.

Further evidence is found in reference AB, Byrnes et al., 1973, *Biochemistry*, 12:4378. At page 4381 in table 3 the authors have provided a list of known DNA synthesis inhibitors including the intercalators, EtBr and Actinomycin D.

Having explained that those of skill would recognize that intercalating fluorophores would likely inhibit polymerase activity, the question of the significance of Sutherland arises. For Sutherland to render the pending claims obvious, the teachings have to be modified according to Mullis. The case law clearly explains when a patent examiner may properly use references to modify prior art teachings. The mere fact that the prior art could be modified to form a claimed combination does not render the claims obvious absent a suggestion in the art to make said modification furthermore, if the modification would render the prior art combination inoperable, the modification is non-obvious. *In re Gordon*, 221 USPQ 1125, 1127 (Fed. Cir. 1984).

Thus according to the patent case law, in order for Sutherland to make out the *prima facie* case of obviousness, the disclosure must overcome the accepted wisdom teaching away and motivate the use of inhibiting agents in PCR. Sutherland falls far short of this hurdle.

In Sutherland at Example 6 (columns 16, 17), the patentees describe a method for measuring DNA polymerase in the presence of fluorescent bibenzimidazole dyes. The assay uses a PCR reaction mixture but does not suggest the use of the dye when conducting PCR. Polymerase inhibition was not a particular concern to Sutherland. If the inhibition was partial but significant, one would never detect the inhibition. The calibration curves would simply adjust for the inhibition.

Moreover, the conditions of the reaction mixture of example 6 are not clearly specified in the text (the buffer solution is ambiguously set forth) and thus even if significant polymerase inhibition were present, a skilled person could not compare the results of Example 6 with the rates depicted in the earlier examples. For this reason, Sutherland cannot be interpreted suggesting the use of such dyes during PCR amplification of DNA. One of skill can only presume that Sutherland's test reactions of Example 6 were significantly inhibited by the presence of the dye, but that use of a calibration chart corrected for inhibition.

In view of the known inhibitory effects of such agents, applicants vigorously assert that the combination of Sutherland and Mullis fails to suggest the claimed invention. The claimed procedures are designed to produce and detect DNA amplification product. Polymerase inhibitors are an anathema to PCR. Even if the inhibitors were not totally disabling to PCR, there is certainly no reason articulated by either Sutherland or Mullis to add them to a PCR reaction mixture prior to thermal cycling. For example, if the inhibition were only 20%, such that the per cycle rate of efficiency were dropped from 2.0 to 1.60, after the typical 30 thermal cycles, the difference in the amount of amplification product would be 95%.

Moreover, clinical and forensic applications of PCR requires reproducible and quantitative results. The inhibition characteristics of the Sutherland agents are unknown. Clearly, Sutherland recognized this fact and describes the use of calibration scales which are customized for each reaction. Based upon the accepted wisdom of the time, there is no reason to conclude that upon repeated thermal cycling that the inhibition rates would be predictable. As explained above, relatively minor degrees of polymerase inhibition produce exponential differences in the final amounts of amplification product. Uncontrollable inhibition would produce irreproducible amounts of PCR amplification product effectively rendering PCR unusable for its most valuable uses.

Having explained in detail that the prior art suggests that fluorescent intercalating agents would inhibit polymerase activity and that artisans familiar with PCR art would not knowingly add inhibitors to their amplification reaction mixtures, applicants believe that the combination of Sutherland and Mullis clearly teaches away from their invention.

(4) Even if the *prima facie* case of obviousness had been properly established, there is evidence of surprising results.

Even assuming that the *prima facie* case of obviousness had been made out, applicants have presented evidence in their specification that the addition of intercalating agents need not inhibit polymerase activity and be present in sufficient quantity to provide the necessary signals. This evidence is found out at example 1 at lines 20-23 which expressly states that no inhibition of PCR was detected at the given concentration of EtBr. In later studies, the results of which were published in Higuchi et al., 1992, *Bio/Technology* 10:413-417 (appended as Exhibit 1) it was demonstrated that concentrations of EtBr between 1/16 and 8.0  $\mu\text{gm/ml}$  of PCR reaction mixture had no noticeable effect on the amount of product produced.

This is a surprising result. Prior to this work, EtBr would have been thought to inhibit DNA polymerase-based

amplification when present at these levels (see Byrne et al.). Moreover, the results are unexpected. There was simply no scientific reason to believe that these agents could be used at non-inhibitory concentrations which were also sufficient to be useful for detecting amplification product.

Applicants have presented four reasons why the pending claims are not obvious. The Examiner has misinterpreted the primary reference, there was no articulation of a convincing line of reasoning as to why one would be motivated to combine the references in the manner of the pending claims, there was an clear teaching away from the invention and finally there were surprising results. Each of the above reasons is legally sufficient to rebut the extant rejection. When combined, applicants submit that the obviousness rejection of claims 1-22 has been rebutted and should be withdrawn.

Claims 10, and 11-16:

The above argument applies broadly to claims 1-22. However a number of the pending claims recite limitations which render the above arguments even more forceful.

Claim 10, as amended, recites detection of amplification product during the amplification process. Measuring the amount of amplification product during amplification has significant benefits to the user and was clearly not suggested by either Mullis or Sutherland each having detected amplification product after the polymerase extensions were complete.

One benefit of simultaneous amplification and detection is that it permits the user to readily plot the thermal cycle step at which the amplification reaction first plateaus (due to a rate limiting reagent). By plotting and comparing the end point of amplification, it is possible to quantitate the starting amount of a target present in a sample.

For this reason and for the general arguments presented for claims 1-22, applicants' urge that claim 10 is patentably nonobvious.

Claim 11 and its dependent claims 12-16 are directed to PCR which refers to exponential amplification involving nucleic acid polymerase and a chain reaction of annealing and denaturing steps. Recitation of PCR renders the above arguments supporting patentability of claim 1 even more forceful. For Sutherland never repeats the DNA polymerase extension process in the presence of the intercalating agents. Thus the above arguments as to the impact of polymerase inhibition are especially applicable to these claims.

Claim 14 is directed to simultaneous amplification and detection of amplification product. Applicants again rely upon the arguments presented above for claims 1-22 and as specifically applied to claim 10.

Claims 12-16:

The Examiner has expressly rejected claims 12-16 as obvious. Claims 12-16 are directed to the use of optic fibers to continuously monitor the fluorescence of a solution. The Examiner finds this art to be old and well known. Applicants rely upon the arguments presented above to rebut this basis for rejecting claims 12-16.

Claims 17-22:

Finally, the Examiner further rejects claims 17-22 as obvious. Claims 17-22 are directed to PCR reaction mixtures having an intercalating agent as a part of the initial PCR buffer. The Examiner has rejected these claims as obvious because the inclusion of the dye within the buffer was motivated by Sutherland. Applicants rely upon the arguments presented above. Specifically, applicants would redirect the attention of the Examiner to the fact that such agents were reported to inhibit polymerase activity and that Sutherland teaches the use of these agents for determining polymerase activity in a non-amplification mode. In view of the reported inhibitory effects of fluorescent intercalating agents, applicants assert that the